multidimensional NMR spectra. Low-resolution structure and dynamics of these tandem domains, along with how these domains react to physical stress, are also discussed.

2577-Pos Board B7
Structural Studies of Obscurin Ig2
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Obscurin (720-900 kD) is a giant sarcomeric signaling protein that plays a crucial role in the arrangement of the basic contractile unit of muscle. Mutations to Obscurin and to Obscurin binding partners have been linked to human muscle diseases such as hypertrophic cardiomyopathies and muscular dystrophy. These diseases likely occur due to the rescindment of specific molecular interactions necessary for suitable function. The modular arrangement of the independently folding domains of Obscurin allows for select analysis of each of these independent binding events. Here, we present the high-resolution crystal structure of the Obscurin Ig2 domain. This region binds to the extreme C-terminus of MBPC-slow variant, although how it does this is unknown. This structure is a canonical Ig-like fold, consisting of two beta sheets coming together to form a beta sandwich.

2578-Pos Board B8
The Unusual Heme Coordination of THB1, a Hemoglobin from Chlamydomonas Reinhardtii
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THB1 is a ~15 kDa ''truncated'' hemoglobin (Hb) found in the cytoplasm of the unicellular green alga *Chlamydomonas reinhardtii*. As for other Hbs within photosynthetic microbes, THB1 is thought to be involved in the management of reactive oxygen/nitrogen species. To complement physiological studies aimed at determining the function of THB1, we are pursuing the characterization of the recombinant protein (rTHB1) [1] with special attention to the heme environment. At pH ~ 5, ferric rTHB1 has an optical spectrum characteristic of a six-coordinate, high-spin complex with a histidine and a water molecule as axial ligands to the heme iron. When the pH is raised near neutrality, the protein undergoes a transition (apparent pKa of 6.5) to a six-coordinate, low-spin complex. Mutagenesis and NMR data collected in the diamagnetic ferrous state provide direct evidence for the ligation of a lysine (K53) on the distal side of the heme [1]. Here, we continue the study of this unusual iron coordination scheme. The distal lysine, like the distal histidine of cyanobacterial globins (GlbNs), facilitates electron transfer. However, unlike the histidine of GlbN, the distal lysine of THB1 does not protect ferric heme from oxidative damage caused by H_2O_2. Analysis of ferrous THB1 NMR data reveals a neutral lysine amino group with highly upfield-shifted ^{15}N signal. Fast-exchange averaging of the two amino protons is a further indication of a dynamic distal coordination. Additional structural information was obtained from diffraction data collected at beamline X25 (National Synchrotron Light Source, Brookhaven National laboratory). Crystals of ferric rTHB1 grown at pH 9.5 diffracted to 1.9 Å resolution ($P6_122$ space group) and the data were used to inspect the structural factors allowing lysine coordination.
[1] Johnson et al. (2014) *Biochemistry* 53:4573
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2579-Pos Board B9
Characterizing Steric Limitations of the Heme Pocket in the Gas-Binding *Tt* H-Nox Protein using Site-Specific Incorporation of Unnatural Amino Acids
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Heme Nitric Oxide and/or Oxygen (H-NOX) binding proteins are bacterial O_2 and/or NO gas-sensing proteins involved in signaling a variety of functions to the cell. Much work has been done to characterize the heme-binding pocket in *Thermoanaerobacter tencongensis* H-NOX (*Tt* H-NOX) using site-directed mutagenesis with the 20 naturally occurring amino acids. We aim to further characterize the heme-binding pocket of *Tt* H-NOX by incorporating unnatural amino acids (UAAs) into the H-NOX scaffold, shedding light on both ligand discrimination and the tuning of ligand affinity. Currently, we are working to understand the steric limitations in this pocket by incorporating halogenated phenylalanine residues and characterizing the spectroscopic, gas-binding, and structural properties of these proteins.

2580-Pos Board B10
Single Molecule Förster Resonance Energy Transfer Studies of the Effect of Deglycosylation on the Structure of Immunoglobulin G
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The deglycosylation of immunoglobulin G (IgG) antibodies with the bacterial enzyme EndoS has been suggested as a potential treatment for some autoimmune disorders as this process leads to a diminished immune response. The reduction in immune response is thought to arise from weakened binding of effector molecules to the fragment crystallizable (Fc) region of IgG antibodies as a result of a conformational change in the Fc region. The nature of this structural alteration is uncertain due to conflicting results obtained from x-ray crystallography and small-angle x-ray scattering studies. To further examine the impact of deglycosylation on the structure of the Fc region, we have examined both glycosylated and EndoS deglycosylated IgG antibodies using single molecule Förster Resonance Energy Transfer (smFRET). The FRET efficiency histograms obtained from studying freely-diffusing, dye-labeled antibodies suggest that the flexibility of the Fc region increases upon deglycosylation.

2581-Pos Board B11
Probing Structural Implications of Unnatural Amino Acid Incorporation into Green Fluorescent Protein
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The ability to study local protein structure and dynamics has been greatly enhanced by the genetic incorporation of unnatural amino acids (UAAs) that contain spectroscopic reporters. An important characteristic of an effective spectroscopic reporter UAA is the ability to probe local protein environments in a relatively non-invasive manner. Here we have investigated the structural consequences of the genetic, site-specific incorporation of the spectroscopic reporter UAA 4-cyano-L-phenylalanine (pCNPhe) into distinct sites in superfolder green fluorescent protein (sfGFP) by X-ray crystallography. This UAA was selected since it can serve as both a vibrational and fluorescent reporter of local protein structure and dynamics. X-ray crystal structures of sfGFP constructs containing pCNPhe will be presented and the structural impacts of the incorporation of this UAA into sfGFP will be discussed.

2582-Pos Board B12
Engineering the Cysteine Motif 'CXXC' into a Protein Imparts It Novel Properties
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We introduced the widely occurring cysteine motif CXXC into the maltose binding protein (one-at-a-time in five alpha-helices, at the N-termini) to test three hypotheses: 1) Does a disulphide bond form at the N-terminus? 2) Does the protein acquire any oxido-reductase activity? 3) Does it acquire new metal-binding properties?
We confirmed: 1) Each cysteine pair forms a stable intrahelical disulphide bond under non-reducing conditions. 2) The five mutant proteins acquire considerable oxidoreductase activity, tested by the insulin aggregation assay. 3) The mutants acquire novel metal-binding properties for Ni^{2+}, Cd^{2+}, and Zn^{2+} upon reduction. We observed that introducing the CXXC motif neither destabilizes the protein nor affects its global structure.
Our results demonstrate that introduction of CXXC motifs can be used to probe alpha-helix start sites and to introduce oxidoreductase and metal binding functionality into proteins.

2583-Pos Board B13
Deciphering the Glycosylation Code
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Asparagine-linked glycosylation, the cotranslational attachment of a carbohydrate to an asparagine sidechain, dramatically impacts protein folding, stability, and structure. However, the ''glycosylation code'' that relates these effects to protein sequence remains unsolved. This work investigates the underlying mechanism of an experimentally observed asx- to beta-turn conformational switch that is induced by the glycosylation of a short peptide using atomistic molecular dynamics simulations. In order to distinguish between the effects of specific and nonspecific interactions with the carbohydrate, these simulations considered model peptides that were N-linked to a disaccharide and also to a steric crowder of the same shape. The simulations suggest that nonspecific steric crowding by the N-linked disaccharide does not significantly alter the peptide free energy surface. However, the combination of steric crowding with specific interactions, e.g. hydrogen bonding and aromatic-glycan stacking,

dramatically impacts the peptide ensemble and stabilizes the conformational switch. Motivated by these results, sequence specific effects of N-linked glycosylation were investigated by additional simulations of peptides with the central sequon Pro-Asn-Gly/Ala-Thr-Trp/Ala. The simulations suggest that sequences with glycine adjacent to the glycosylation site readily form compact beta-turns upon glycosylation, while sequences with alanine in this location appear to be much less influenced by the glycan. Somewhat surprisingly, the simulations suggest that aromatic-glycan interactions are less significant for this conformational switch. In order to corroborate these simulations, structural analysis of a dataset of glycoprotein structures was performed. This analysis indicates that the simulations of short glycopeptides are quite predictive of the conformations adopted by glycosylated sequences in the context of full length glycoproteins.

2584-Pos Board B14

Interactions between Thrombomodulin and Complement Component C3 Studied by Fluorescence Resonance Energy Transfer

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Interactions between the lectin-like domain of thrombomodulin (TMD1) and the complement system may provide a link between coagulation and inflammation. Thrombomodulin is an integral membrane protein that is most studied for its role as a down-regulator of blood clotting, but the lectin-like domain has more recently been shown to interfere with complement proteins within the innate immune system. Of these complement proteins, specifically component 3 (C3) has been shown to interact with TMD1. C3 is at the center of three different modes of activation of the complement system and may provide a good target for regulation of the system. By understanding the interactions between TMD1 and C3, we would be able to target the causes of many inflammatory diseases. C3 has been isolated and purified from bovine blood plasma, and TMD1 was expressed in Pichia pastoris and then purified. The stability of both TMD1 and C3 was studied using urea-induced unfolding, revealing intrinsic tryptophan fluorescence. After labeling TMD1 with a fluorophore and C3 with a quencher, interactions were studied using Fluorescence Resonance Energy Transfer (FRET). The use of FRET will not only verify that the two proteins are interacting, but will also eventually allow for the calculation of binding constants to measure the binding affinity of TMD1 and C3. Future studies will include using protein pull-down assays and hydrogen/deuterium exchange coupled with mass spectrometry to further study the interaction.

2585-Pos Board B15

Structural Analysis of the Ectodomain of the Anti-Viral Protein BST-2

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Human BST-2/tetherin is a host factor that inhibits release of HIV-1, HIV-2, and SIV from the cell surface. Viruses can evade this inhibition through antagonistic viral protein interactions with BST-2. Structurally, full-length BST-2 consists of an N-terminal cytoplasmic domain, a transmembrane domain, an ectodomain, and a C-terminal membrane anchor. The N-terminal side of the ectodomain contains three cysteine residues; each can contribute to the formation of cysteine-linked dimers. We explored the ectodomain of BST-2 to further understand the flexibility of the protein as it relates to function. Recent cellular studies suggest BST-2 is flexible with regards to the dimerization and ability to function properly. However, X-ray crystallography suggests the ectodomain is rigid. Through limited proteolysis, molecular dynamics and small-angle x-ray scattering, we showed that the ectodomain of BST-2 is flexible. However, the flexibility of the membrane bound BST-2 and the interaction between the HIV-1 viral antagonist protein, Vpu, is still unknown. To investigate the flexibility and the interaction between BST-2 and Vpu, we are optimizing conditions for purifying and crystallizing the full-length BST-2, Vpu and the BST-2/Vpu complex. These studies will show how the innate immune system protein, BST2, interferes with viral budding.

2586-Pos Board B16

Macromolecular Crowding of a Protein Complex by Small Angle Neutron Scattering and Small Angle X-Ray Scattering

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Macromolecular crowding can alter the structure and function of biological molecules. We used small angle scattering to measure the change in size of a protein complex induced by macromolecular crowding. Crowding of the homodimer, superoxide dismutase (SOD) was induced using polyethylene glycol - 400, triethylene glycol, methyl-α-glucoside and trimethylamine N-oxide. Parallel small angle neutron scattering (SANS) and small angle x-ray scattering (SAXS) allowed us to unambiguously attribute apparent changes in radius of gyration to changes in the structure of SOD. We find that SOD is highly compressible for changes in volume up to 9%. Resistance to deformation beyond 9% increased dramatically.

2587-Pos Board B17

Conformational Dynamics and Functional Asymmetry of ABCE1, a Ribosome Recycling Protein

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Ribosome recycling is enabled by ABCE1, a twin-ATPase protein attached to an iron sulfur (FeS) cluster. ABC proteins typically adopt an ATP-bound closed state and an open state, however, for ABCE1, only the structure of an open or a semi open state are experimentally resolved. Furthermore, despite the structural similarity of the two nucleotide binding domains (NBDs), they were reported to show functional asymmetry in mutations of the functional glutamates in both NBDs. In our study we have modeled the ATP bound conformation relying on the similarity to other ABC proteins combined with essential dynamics simulations. We then used thermodynamic cycles to explore interface mutations that shift the equilibrium towards a closed conformation in order to guide experiments and stabilize the protein in its closed state so that a better basis can be provided for its crystallization. In addition, we examine the question of whether the asymmetric function of ABCE1 observed in mutations at parallel positions in the NBDs might stem from the change in its open-closed equilibrium.

2588-Pos Board B18

Moving Macromolecular Surfaces under Hydrophobic/Hydrophilic Stress

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Hydrophobic and hydrophilic interactions can be described as dispersive interactions throughout the molecules, interaction between permanent or induced dipoles and ionic interactions. Hydrophobic effect is synonymous with dispersive interactivity and hydrophilic one is synonymous with polar interactivity. Unification of all these interactions in one interaction is electromagnetic interaction's dependence on interacting body's geometries. Hydrogen bonding is direct implication of such geometric dependence. Given the uniqueness of the problem, which is obvious for two surfaces, we only focus on two-dimensional surfaces embedded in the higher four-dimensional Minkowskian ambient space. Though, the analysis can be easily extended to hypersurfaces of any dimension. Limitation by two surfaces, embedded in four space-time, which is necessary to describe electromagnetism, is consequence of specificity of processes that takes place on macromolecular surfaces. In the following paper we discuss equations for the dynamic of macromolecular surfaces under the influence of potential energy consisting from four-potential time four-current and contraction of electromagnetic tensor. The macromolecular surfaces are modeled as a two-dimensional surface with a variable surface mass density. Kinetic energy is calculated according to calculus of moving surfaces. Definition of Lagrangian by subtracting potential energy from kinetic energy and setting minimum action principal are yielding nonlinear equations for moving surfaces under hydrophobic-hydrophilic interactions. The equations can describe uniqueness and specific functionality of proteins and nucleic acids. Shape minimization problems as well as minimum surface problems are also discussed.

2589-Pos Board B19

Dynamics of Gal80p in the Gal80p-Gal3p Complex Differ Significantly from the Dynamics in the Gal80p-Gal1p Complex: Implications for the Higher Specificity of Gal3p Towards Transcriptional Induction of Gal Genes

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Expression of the GAL gene in Sacharomyces cerevisiae is regulated by three proteins; Gal3p/Gal1p, Gal80p and Gal4p. Both Gal3p and Gal1p act as transcriptional inducers, though Gal3p has a higher activity than Gal1p. The difference in activity may depend on the strength of the interaction and dynamical behavior of these proteins during complex formation with the repressor protein Gal80p. To address these queries we have modeled the binding interface of the Gal1p-Gal80p and Gal3p-Gal80p complexes. The comparison of the dynamics of these proteins in the complex and in the Apo protein was carried out. It was observed that the binding of Gal3p with Gal80p induces significant flexibility in Gal80p on a surface different from the one involved in binding with Gal3p. Several other differences at the interface between the Gal3p-Gal80p and the Gal1p-Gal80p complex were observed, which might permit Gal3p to act as a transcriptional inducer with higher activity. Further, on the basis of our finding